

<https://helda.helsinki.fi>

Genetic modifications of horticultural plants by induced mutations and transgenic approach

Suprasanna, P.

International Society for Horticultural Science
2017

Suprasanna , P , Ganapathi , T R , Ghag , S B , Jain , S M , Lambardi M. , A-S A A (ed.) & J.M. , A-K (ed.) 2017 , Genetic modifications of horticultural plants by induced mutations and transgenic approach . in A A Abul-Soad , J M Al-Khayri & M Lambardi (eds) , IX International Symposium on In Vitro Culture and Horticultural Breeding . Acta Horticulturae , vol. 1187 , International Society for Horticultural Science , Leuven , pp. 219-232 , International Symposium on In Vitro Culture and Horticultural Breeding , Giza , Egypt , 11/01/2016 . <https://doi.org/10.17660/ActaHortic.2017.1187.22>

<http://hdl.handle.net/10138/309412>

<https://doi.org/10.17660/ActaHortic.2017.1187.22>

publishedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Genetic modifications of horticultural plants by induced mutations and transgenic approach

P. Suprasanna¹, T.R. Ganapathi¹, S.B. Ghag² and S.M. Jain^{3,a}

¹Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, 400085, India; ²UM-DAE Centre for Excellence in Basic Sciences, Kalina, Santa Cruz, Mumbai-400098, India; ³Dept. Agricultural Sciences, University of Helsinki, PL-27, Helsinki, Finland.

Abstract

Climate change has pressed the need to develop improved horticultural crop cultivars capable of tolerating extreme environmental conditions besides sustaining yield and quality. Induced mutations provide a viable option for the generation of a novel genetic variation. In horticultural crops, more than 800 mutant cultivars have so far been developed, and a majority of them are ornamentals followed by vegetables, fruits, decorative trees, berries, nuts, ornamentals and other plants. Transgenic technology has also led to the improvement of horticultural crops for plant type, fruit-shelf life, floral and quality assets besides resistance to biotic and abiotic stresses. Mutagenesis techniques have been integrated with in vitro culture and other molecular biology technologies, such as molecular markers and high-throughput mutation screening, thereby becoming more powerful and effective in crop breeding.

Keywords: induced mutations, horticultural crops, mutant cultivars, transgenic plants, crop improvement

INTRODUCTION

The importance of agricultural research and food security remains critical as global population will attain nine billion people in 2050. The increased human population growth will greatly impact our present and future agricultural practices and would require innovative approach for sustainability. Therefore, improving plant productivity may lead to reduce expansion of cultivated land (Lal, 2004a). There are multiple challenges such as climate change and extreme weather events exacerbate the agriculture system and that may have adverse impact on plant genetic resources, genetic diversity and erosion, and global food security (Ahuja et al., 2010; Ahuja and Jain, 2015, 2016). Adaptation to climate change and continuity of sustainability, major interventions are required to transform current agricultural practices (Beddington et al., 2012). Environmental stress factors such as drought, elevated temperature, salinity and rising CO₂ affect plant growth and pose a growing threat to sustainable agriculture. To cope up climate change catastrophes, plants have evolved a defense mechanism at the physiological, genetic and molecular levels to adapt accordingly. Such insight is required to breed crops, isolate mutants, develop novel plant types or produce transgenic cultivars with enhanced tolerance to multiple environmental stress factors.

The use of induced mutations has played a key role in the improvement of superior plant cultivars (Ahloowalia and Maluszynski, 2001; Maluszynski et al., 2004; Jain, 2005). The frequency spontaneous mutation rate is very low (1×10^{-6}), and can't be used in genetic improvement of plants. Induced mutations with physical and chemical mutagens enhance the mutation rate by several folds to enable plant breeders to incorporate in plant breeding programs to develop new plant cultivars with useful desirable traits. Moreover, a single induced mutant can have several traits, e.g. disease resistance, high yield, plant architecture, and abiotic stress. This is in contrast to transgenic plants techniques, where in single gene trait is expressed.

A large number of improved mutant cultivars have been released for commercial

^aE-mail: mohan.jain@helsinki.fi



cultivation in different plant species demonstrating potential of the mutation breeding in crop breeding (Jain, 2005; Kharkwal and Shu, 2009; Jain and Suprasanna, 2011; Suprasanna et al., 2015). Induced mutations provide a viable option for the generation of a novel genetic resource. These can have either one or a few characters modified in an otherwise promising cultivar without significantly altering the remaining (and often unique) genetic background. The horticultural plant species are either sexually or asexually or vegetatively propagated and that makes cross-breeding often difficult due to long vegetative phase, high heterozygosity and polyploidy, incompatibility including apomixis and sterility. By induced mutations, these barriers could be taken care for crop improvement (Suprasanna and Nakagawa, 2012). Mutagenesis techniques have also been complemented with in vitro culture and other molecular biology technologies, such as molecular markers and high-throughput mutation screening, thereby offering more powerful and effective tools in crop breeding (Shu, 2009; Parry et al., 2009).

Plant genetic engineering techniques offer newer opportunities for horticultural plants (Mou and Scorza, 2011; Xiong, 2015). They are more relevant and feasible when cultivars often cannot be bred through hybridization or mutagenesis, and unavailability of gene pool for traits of interest in the natural population (Chandler, 2013). Transgenic plants are also referred as genetically modified (GM) plants, and well suited to incorporate desirable genes into elite cultivars for improving traits including insect and pathogen resistance, stress tolerance, nutritional status, quality, flower morphology, color, plant architecture, ripening etc. Biotechnological approaches including transgenic techniques are promising in improving yield, agronomic traits, product quality and novel uses of horticultural products (Nishihara and Nakatsuka, 2010; Hallerman and Grabau, 2016).

In this article, we present an overview of induced mutations and transgenic technologies for the improvement of horticultural crops.

INDUCED MUTATIONS

The term mutagenesis applies to methods used for the induction of mutations in plant cells to improve agronomical valuable traits in well-adopted cultivars. Most mutagenic plant populations are generated by treating seeds or other plant propagules with physical mutagens including X-rays, Gamma rays, alpha particles, fast neutrons, UV and cosmic rays; and chemical mutagens: sodium azide, ethyl methanesulphonate, methyl methanesulphonate, hydroxylamine and N-methyl-N-nitrosourea. Irradiation usually results in large-scale deletions while chemical mutagens cause point mutations. Ion beam radiation and space mutagenesis are relatively recent and have generated great interest in the induction of mutations. Ion beams produced by a cyclotron deposit high energy on a target and the linear energy transfer (LET) ranges from 22.5 keV μm^{-1} to 4000 keV μm^{-1} compared to γ -rays and X-rays (Ryuto et al., 2008). It is well known that high-LET radiation shows stronger biological effects than low-LET radiation.

Aerospace environment with strong features of cosmic radiation, microgravity, weak geomagnetic field has been employed to induce mutations. The space-induced mutations are induced in the space environment, which is either achieved by carrying plant material (seeds or plant propagules) in a recoverable spacecraft (satellites and space shuttles) or high altitude balloons to generate novel plant germplasm (Liu et al., 2007a). China has officially approved 60 new cultivars of rice, wheat, cotton, sesame, pepper, tomato and alfalfa with improved characters of high yield, good quality and multiple resistance including some useful rare mutations (Liu et al., 2007b).

INDUCED MUTATIONS AND ECONOMIC BENEFITS

The International Atomic Energy Agency (IAEA) with the Food and Agriculture Organization (FAO) of the United Nations (UN) has maintained 'Mutant Variety Database' which catalogues officially released mutant crop cultivars. There are presently over 3200 officially released mutant cultivars in over 170 species (FAO/IAEA MVDB, 2016). While the highest number of varietal entries is of cereals, improvement of major traits includes resistance to biotic and abiotic stresses, improved quality and yield, plant architecture and

maturity. The economic impact of mutant cultivars has been well documented suggesting that mutant cultivars are contributing millions of dollars annually to local economies (Ahloowalia et al., 2004; Jain, 2005). It is estimated that mutant cultivars provided about \$804 million and \$11.2 billion to the economies of Japan and the US, respectively, in the late 1990's (Kume et al., 2002). Among horticultural plants, two cultivars of peppermint, 'Todd's Mitcham' and 'Murray Mitcham,' have become popular among consumers; Japanese pear fruit (*Pyrus pyrifolia*) 'Gold Nijisseiki' and grapefruit 'Rio Star' are grown in expanded production area (Mba et al., 2009).

MUTAGENESIS IN HORTICULTURAL PLANTS

Over 800 mutant cultivars of horticultural plants have been released and majority of them are ornamentals for floral traits followed by vegetables, fruits, decorative trees, berries, nuts, ornamentals and other plants (FAO/IAEA MVDB, 2016; Figure 1A). Mutation breeding has been highly successful in ornamental plants e.g. changes in phenotypic characteristics like color, shape or size of flower and chlorophyll variegation in leaves can be readily detected (Jain, 2006; Datta, 2009). In addition, the heterozygous nature of many ornamentals results in high mutation frequency rate. Gamma radiation treatment has been highly effective in inducing useful desirable mutations in the development of several mutant cultivars of the ornamental plants. In horticultural plants, mutagenesis combined with management of chimera and in vitro mutagenesis are the most promising methods for developing new and novel cultivars. Chronic gamma radiation over acute irradiation has resulted in a number of useful mutant cultivars (Jain et al., 2010; Datta, 2014). In vegetable crops, tomato tops the list with 27 mutant cultivars. In crops like banana and date palm, somatic embryogenic cultures have been mostly irradiated for the isolation of disease resistant mutants. For example, in date palm, several putative mutants tolerant to Bayoud disease were isolated (Jain, 2012). The IAEA mutant cultivar database indicates that fruit and vegetable mutants showed improved maturity, product quality, plant architecture besides disease resistance and yield (Figure 1B). So far, induced mutations have improved fruit crop traits such as plant size, blooming time and fruit ripening, fruit color, self-compatibility, self-thinning, and resistance to pathogens. To date, 55 mutant cultivars have been developed in some economically important fruit crops like apple, banana, Strawberry, orange, mandarin, pomegranate, grape, grapefruit etc. In banana, mutants for several traits including reduced height, tolerance to Fusarium wilt, early flowering, large fruit size and black Sigatoka tolerant types were isolated (Jain, 2002, 2010; Jain et al., 2011).

Among ornamentals, Chrysanthemum dominates as the major ornamental plant with more than 250 entries as listed mutants followed by rose with 67 entries (FAO/IAEA MVDB, 2016). Some notable mutant cultivars in ornamentals include *Amaryllis*, *Bougainvillea*, *Canna*, *Chrysanthemum*, *Dahlia*, *Gerbera*, *Gladiolus*, *Hibiscus*, hybrid lily, *Tagetes* sp., rose, tuberose, *Narcissus* sp. etc., have been released (Datta, 2009). In orchids, the released mutant cultivars predominantly showed better plant type, growth patterns, floral color and shape (Table 1). In Malaysia, several new cultivars of ornamental have been officially released: *Hibiscus rosa-sinensis* 'Siti Hasmah PinkBeauty', 'Siti Hasmah RedShine' and 'Nori', *Cordyline terminalis* 'Teguh', 'Jaguh' and 'Mantap', *Cordyline fruticosa* 'Shuhaii', *Duranta repens* 'Marginata' and 'Variegata', orchids (*Dendrobium* 'Sonia KeenaRadiant', 'Sonia KeenaOval', 'Sonia KeenaAhmadSobri' and 'Sonia KeenaHiengDing') and *Petunia hybrid* 'NK Tropicana' (Ahmad et al., 2012). Figure 2A shows chrysanthemum mutants. In *Zinnia elegans* 'Dreamland', seeds irradiated with 75 and 100 Gy gamma rays showed interesting mutants for flower form, color (Figure 2B) in M3 generation (Pallavi et al., unpublished results).

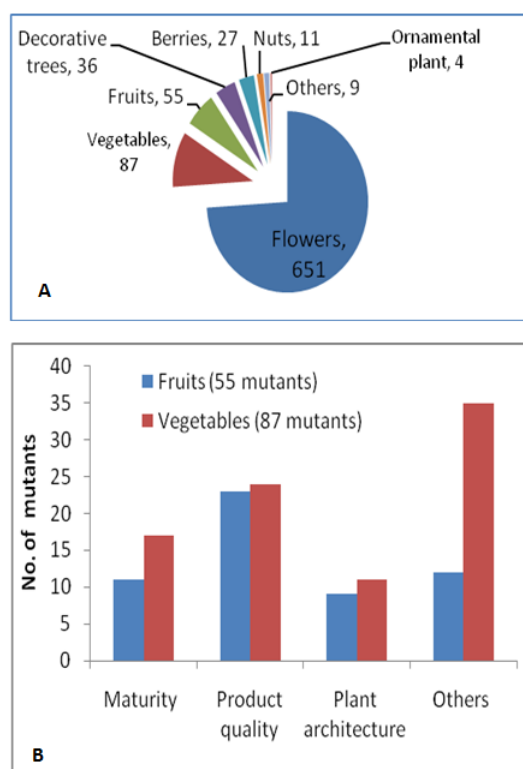


Figure 1. A. Mutant cultivars in horticulture plants. B. Categorization of fruit and vegetable mutants based on improved traits (others: yield/disease resistance traits). Based on FAO/IAEA MVDB (2016).

Table 1. Mutant cultivars of orchids. Based on FAO/IAEA MVDB (2016).

Cultivar name	Latin name	Common name
Cocktail Dress	<i>Cymbidium</i> sp.	Boat orchids
Dong-i	<i>Cymbidium</i> sp.	Cymbidium
Gold One	<i>Oncidium</i> sp.	Orchid
Sherley Baby White	<i>Oncidium</i> sp.	Orchid
White Wings	<i>Alstroemeria</i> sp.	Alstroemeria



Figure 2. A. Gamma ray induced mutants of *Chrysanthemum*. B. Gamma ray induced mutants of *Zinnia elegans* (courtesy: Prof. Leo D'Souza, Laboratory of Applied Biology, St. Aloysius College, Mangalore, India).

INDUCED MUTATIONS AND TRANSGENICS: COMPARISON AND PRECEDENCE

Both induced mutations and transgenic approaches have been successfully applied for the improvement of crop plants. Induced mutations can be used to change or alter one character of an elite cultivar, for example, reduction in plant height without compromising the yield, resistance to diseases and pests, salinity, drought stress tolerance, metal toxicity, reducing the anti-nutritional factors etc. Seeds and other vegetative propagules or in vitro meristematic tissue can be used as the target material for mutagenesis.

Transgenics is a precise gene transfer method, used for transferring trait-specific gene or a transcription factor. However, plant regeneration by tissue culture and genetic transformation, or in planta transformation protocols are essential for gene transfer in any cultivar (Katavic et al., 1994; Clough and Bent, 1998; Trieu et al., 2000; Kumar et al., 2011; Andrieu et al., 2012). In induced mutagenesis, prior knowledge of genome or the gene sequence information is not required as it is random and chance of getting the desirable mutant depends on screening of large populations subsequent to mutagen treatment. Recently, molecular markers have been developed to identify the traits at an early stage so that the number of plants screened can be reduced by saving both time and cost. For transgenics, prior knowledge of gene sequence is a prerequisite for cloning and over expression in transgenic lines quickly to enable screening for the desirable trait. While induced mutations are subjected to diplontic and haplontic selection, transgenics are subjected to position effect and silencing (Mba et al., 2009). There are major concerns of biosafety and regulatory approvals to release a transgenic cultivar, whereas induced mutants don't require such approval. The other limitations of transgenics include mutations induced through the transformation process in some of the commercialized transgenic crops (Wilson et al., 2006) which can be minimized by using in planta transformation (Bent, 2000), avoiding in vitro dedifferentiation steps during transformation, use of linearized gene cassettes instead of using plasmids to avoid the plasmid backbone integration in biolistic gene transformation method.

TRANSGENIC METHODS AND EXAMPLES

Vegetables and fruits are grown worldwide and provide us with the daily requirements of vitamins, minerals and dietary fibre. These crops suffer from abiotic stresses, nutritional imbalances and incur losses due to pest and diseases. Transgenic approaches render plant biotechnologists to insert desirable traits into elite cultivars. Single or multiple genes can be introduced stably into cultivars by available transformation protocols. Several vegetable and fruit crops are genetically modified to achieve resistance to insects, fungi, bacteria and viruses, tolerance to environmental stresses (drought, cold and salinity) and for improving quality traits such as nutritional content, flavor and shelf life (Xiong et al., 2015) (Table 2). Managing viral diseases is challenging as it cannot be controlled by application of chemicals. Papaya ringspot disease caused by Papaya ringspot virus (PRSV) is the most debilitating disease that caused severe destruction of papaya plants in late 20th century. Transgenic papaya plants expressing the coat protein gene of PRSV were resistant to PRSV infection and have been used in Hawaii to control PRSV (Gonsalves, 2004; Tripathi et al., 2007). The most distressing fungal disease is the potato late blight disease caused by *Phytophthora infestans* and its resistance genes were identified in wild relatives and introduced into cultivated potato plants by genetic manipulation (GM) technology to impart blight resistance (Haltermann et al., 2008; Jones et al., 2014; Jo et al., 2014).

Table 2. Transgenic technology for improvement of vegetable and fruit crops. Source: FAO/IAEA MVDB (2016).

Plant	Source	Gene	Character	Reference
Tomato	Arabidopsis	<i>NPR1</i>	Disease resistance	Lin et al., 2004
	Sweet pepper	<i>pflp</i>	Disease resistance	Huang et al., 2007
	Human	cathelicidin	Disease resistance	Jung, 2013
	Sweet pepper	<i>Bs2</i>	Disease resistance	Horvath et al., 2012
	Tomato yellow leaf curl virus	Intergenic region, coat protein gene, V2 gene and replication-associated gene	Disease resistance	Ammara et al., 2015
	Rice and Alfalfa	<i>CHI</i> and <i>alfAFP</i>	Disease resistance	Chen et al., 2009
	Tomato	<i>THT</i>	Disease resistance	Campos et al., 2014
	Yeast	<i>HAL1</i>	Salt tolerance	Gisbert et al., 2000
	Tomato	<i>SISHN1</i>	Drought tolerance	Al-Abdallat et al., 2014
	<i>Erwinia uredovora</i>	<i>CrtI</i>	Enhanced carotenoid	Römer et al., 2000
Potato	Tomato	α -Man and β -Hex	Enhanced shelf life	Meli et al., 2010
	Tomato	Polygalacturonase	Enhanced shelf life	Krieger et al., 2008
	<i>Solanum venturii</i>	<i>Rpi-vnt1.1</i>	Disease resistance	Jones et al., 2014
	Potato virus X and Potato virus Y	Coat protein sequence of and the nuclear inclusion protein	Disease resistance	Bai et al., 2009
	<i>Aspergillus niger</i>	Glucose oxidase	Disease resistance	Wu et al., 1995
	<i>Solanum bulbocastanum</i>	<i>RB</i>	Disease resistance	Song et al., 2003; Halterman et al., 2008
	Potato virus Y	Coat protein	Disease resistance	Missiou et al., 2004
	<i>Pleurotus sajor-caju</i>	<i>GPD</i>	Salt tolerance	Jeong et al., 2001
	<i>Arabidopsis thaliana</i>	<i>CBF1</i>	Drought tolerance	Nichol et al., 2015
	<i>Amaranthus hypochondriacus</i>	<i>AmA1</i>	Nutritive value	Chakraborty et al., 2000
Eggplant	<i>Petunia hybrida</i>	<i>DFR</i>	Nutritive value	Kostyn et al., 2013
	Alfalfa	Glucanase	Disease resistance	Singh et al., 2014
	<i>Bacillus thuringiensis</i>	<i>cryIIIA</i>	Insect resistance	Jelenkovic and Billings, 2000
Cabbage	Tomato	<i>Mi-1.2</i>	Pest resistance	Goggin et al., 2006
	Bacteria	<i>mtlD</i>	Abiotic stress tolerance	Prabhavathi et al., 2002
	<i>Aspergillus niger</i>	Glucose oxidase	Disease resistance	Lee et al., 2002
Banana	Human	Cathelicidin	Disease resistance	Jung et al., 2012
	<i>Bacillus thuringiensis</i>	<i>cryIIa8</i>	Insect resistance	Yi et al., 2016
	<i>Petunia</i>	<i>PhDef1/ PhDef2</i>	Disease resistance	Ghag et al., 2012
Banana	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i>	Velvet protein gene or <i>FTF1</i>	Disease resistance	Ghag et al., 2014
	Sweet pepper	<i>Hrap</i> or <i>pflp</i>	Disease resistance	Tripathi et al., 2011
	Banana bunchy top virus	<i>Rep</i>	Disease resistance	Shekhawat et al., 2012
	Rice	chitinase	Disease resistance	Kovács et al., 2013
	Banana	<i>MusaDHN-1</i>	Abiotic stress tolerance	Shekhawat et al., 2011
	Banana	<i>MusaPIP1;2</i>	Abiotic stress tolerance	Sreedharan et al., 2014
	Soybean	Ferritin	Increased nutritional content	Kumar et al., 2011
Papaya	<i>Papaya ringspot virus</i>	Coat protein gene	Disease resistance	Tripathi et al., 2007
	Papaya	<i>ACO1</i> and <i>ACO2</i>	Enhanced shelf life	Sekeli et al., 2014
Plum	<i>Plum pox potyvirus</i>	Coat protein gene	Disease resistance	Scorza et al., 2001
Squash	<i>Cucumber mosaic virus</i> , <i>Watermelon mosaic virus 2</i> or <i>Zucchini yellow mosaic virus</i>	Coat protein genes	Disease resistance	Tricoll et al., 1995
	<i>Squash mosaic comovirus</i>	Coat protein gene	Disease resistance	Pang et al., 2000
Apple	<i>Malus x domestica</i>	<i>NPR1</i>	Disease resistance	Malnoy et al., 2006
	<i>Malus floribunda</i> 821	<i>Vfa1</i> and <i>Vfa2</i>	Disease resistance	Malnoy et al., 2008
	<i>Malus hupehensis</i>	<i>NPR1</i>	Disease resistance	Chen et al., 2012
	<i>Agrobacterium tumefaciens</i>	<i>iaaM</i> , <i>iaaH</i> , and <i>ipt</i>	Disease resistance	Viss et al., 2003
	T4 bacteriophage and <i>Hyalophora cecropia</i>	<i>T4L</i> and <i>attE</i>	Disease resistance	Ko et al., 2002
	<i>Malus x domestica</i>	<i>MdcMDH</i>	Abiotic stress tolerance	Wang et al., 2016
	Spinach	<i>SOD</i>	Abiotic stress tolerance	Macarasin et al., 2008
	Apple	Polyphenol oxidase	Improved quality	Strauss et al., 2011

Another successful demonstration is ‘HoneySweet’ plum plants resistant to Sharka disease. RNA interference technology was used to deliver effective, stable, durable and

heritable resistance in transgenic plum plants and, after extensive testing and risk assessment of 'HoneySweet' in laboratory, greenhouse and in the field for over 20 years it has been cleared for cultivation in the USA (Scorza et al., 2013). Transgenic tomato plants were genetically engineered to resist insect pests by expressing *Bacillus thuringiensis* (Bt) *cry1Ab* gene (Koul et al., 2014) or protected against several viruses by using RNA interference technology (Fuentes et al., 2016). Genetically modified eggplant (*Solanum melongena*) expressing *Bacillus thuringiensis cry1Ac* gene provided high level resistance to fruit and shoot borer which was difficult to control by non-GM methods. Bt-brinjal is a successful example in the South-East Asia where brinjal is a popular vegetable, and may go for commercial release (Krishna and Qaim, 2008; Randhawa et al., 2012).

Banana is threatened by Fusarium wilt disease (Panama disease) caused by a soil-borne root pathogen *Fusarium oxysporum* f. sp. *cubense* [Foc] (Ghag et al., 2015). Since banana plants are propagated vegetatively, traditional breeding practice can't be undertaken to introduce resistance trait. Transgenic banana plants engineered to express defense peptides (Chakrabarti et al., 2003; Ghag et al., 2012) or Foc specific double stranded RNAs (Ghag et al., 2014) demonstrated enhanced resistance to Fusarium wilt disease. The banana aphid-transmitted Banana bunchy top virus (BBTV) is the most destructive viral pathogen of bananas and plantains, and lack of natural sources of resistance has necessitated the use of transgenic technologies for obtaining BBTV-resistant banana cultivars. Shekhawat et al. (2012) have successfully developed BBTV-resistant transgenic banana plants via RNA interference approach (Figure 3) by using intron-hairpin-RNA (ihpRNA) transcripts corresponding to viral master replication initiation protein.



Figure 3. Transgenic banana plants engineered for banana bunchy top virus resistance using replicase RNAi construct. Left: Control plant showing the symptoms after bioassay. Right: healthy transgenic plant after bioassay.

Although transgenic technology has more focused on the improvement of horticultural crops to resist diseases or insects, abiotic stress factors continue to impose limitations on yield and productivity. Under the climate change, there is an urgent need to develop horticultural cultivars capable of tolerating extreme environmental conditions. Therefore, climate-responsive and drought tolerant transgenic plants can be useful for judicious management of water and can increase the total acreage under production. Transgenic

potato lines containing the *AtCBF1* gene under the control of the stress-inducible promoter *COR15a* were field evaluated for four years and results showed that the transgenic lines were better endured to tolerate drought conditions (Nichol et al., 2015). Transcription factors such as DREB (dehydration-responsive element-binding proteins) and WRKY (WRKYGQK motif and a zinc finger region) when expressed under the control of an inducible promoter have shown multiple stress (cold, salt and drought) tolerance in grape and banana (Jin et al., 2009; Shekhawat et al., 2011).

GM technology can also deliver genes from different sources directly into the local cultivars to increase the levels of vitamins and/or mineral nutrients thereby increasing the nutritional content of the food. Vitamin A deficiency is a serious problem worldwide causing infant mortality and clinical conditions such as partial to complete blindness. Enriching and accumulating vitamin A precursor carotenoid has been achieved in few important fruit and vegetable crops like banana (Waltz, 2014) and potatoes (Diretto et al., 2007) by stacking genes namely phytoene synthase, phytoene desaturase and lycopene beta-cyclase. Inorganic nutrients or minerals are equally important and must be essentially considered in fortification programs to overcome deficiencies leading to prevent threat to human life. GM technology focuses on the enhancement of mineral nutrients in staple crops either by increasing the solubility or mobilization into plants, trafficking and storage in the edible organs and increasing the bioavailability of these minerals. Studies are underway to increase iron, zinc and calcium content in crops such as potatoes, banana and carrot (Pérez-Massot et al., 2013).

Transgenic technology is useful in extending/increasing the shelf- life of fruits and vegetables by regulating the genes responsible for ripening. N-glycoprotein modifying enzymes, α -mannosidase and β -D-N-acetylhexosaminidase suppression in transgenic tomatoes resulted in enhanced fruit shelf- life by 30 days (Meli et al., 2010). However transgenic tomato plants engineered to suppress the activity of polygalacturonase, ACC oxidase and ACC synthase enzyme demonstrated delay in ripening process and thereby increased shelf- life (Gupta et al., 2013). Delayed fruit ripening was achieved in papaya, apple, melon and banana by repressing either the ACC synthase or MADS-box genes (Ayub et al., 1996; Tecson Mendoza et al., 2008; Schaffer et al., 2013; Elitzur et al., 2016).

Tomato has been extensively used for genetic manipulation studies. The traits incorporated include delayed ripening, disease resistance, insect pest tolerance, chilling, salinity, drought tolerance nutritional quality improvement, fruit color and pharmaceuticals (Hefferon, 2015). Genetically modified 'Flavr savor' tomato commercialized in 1994 was the first transgenic tomato plant developed for delayed softening of fruits by suppressing polygalacturonase gene by antisense technique. Tomatoes have also been engineered to produce more antioxidants and animal feeding trials showed the reduction of cancer incidence (Butelli et al., 2008). Recently, Fatima et al. (2016) studied the metabolomic profile of transgenic tomatoes, grown in different field growing conditions like conventional tillage, surface mulching with polyethylene sheets, and with remains of cover crop vegetation and found that the change in metabolite concentrations in the transgenic plants similar to traditionally grown plants.

In ornamentals, production of flowers and potted plants offers economic benefit to some developing countries in South America, the Middle East, Asia, and Africa. The global value of the ornamental industry covering production value of ornamentals and the value addition of products is estimated to be 250-400 billion USD (Chandler and Sanchez, 2012). Transgenic research has focused on creating a wider range of flower colors through manipulating either anthocyanins (red and blue colors) or carotenoids (yellow and orange colors), mainly to produce novel colors and to produce natural dyes for industrial purpose (Lu et al., 2003). Transgenic ornamental plants like *Petunia*, *Begonia*, *Torenia* with reduced ethylene activity exhibited longer shelf-life (Olsen et al., 2015). Some recent examples of transgenic ornamentals include, *Cyclamen persicum* (cyclamen), *Gentiana triflora* (Japanese gentian), *Lotus japonicus*, *Phalaenopsis* spp. (phalaenopsis), *Torenia hybrida* (torenia) and *Tricyrtis* spp. (toad lily). The flower color of carnation was modified by overexpression of heterologous flavonoid 3',5'-hydroxylase gene which produces delphinidin derivatives

(Fukui et al., 2003). They characterized the pigments responsible for the shift in flower color to blue in transgenic plants. In *Pelargonium*, transgenic plants transformed with pSAG12::ipt (for auto-regulated cytokinin biosynthesis) showed delayed leaf senescence, increased branching and reduced internodal length (García-Sogo et al., 2012). In addition, leaves and flowers of transgenic plants were intensely colored and reduced in size.

Transgenic carnations, *Petunia* and chrysanthemum overexpressing sense or antisense copy of genes involved in flavonoid biosynthesis pathways resulted in variegated flowers which were field tested and commercialized (Meyer et al., 1987; Courtney-Gutterson et al., 1994; Fukui et al., 2003; Debener and Winkelmann, 2010). Murata et al. (2015) reported the development of transgenic *petunia* plants overexpressing iron (III) phytosiderophore transporter gene and observed the deepening of the flower color and also the plants grew better under saline conditions. Another dimension of transgenic approaches has been the modification in the architecture of plants and/or flowers, flowering time, response to day length, and post-harvest life of potted plants and cut flowers (Chandler and Sanchez, 2012; Olsen et al., 2015). So far, two GM ornamental plants, *D. caryophyllus* and *Rosa hybrida* with modified flower color are commercialized (Dobres, 2011).

CONCLUSIONS

Plant improvement strategies depend on the exploitation of natural or induced genetic diversity. The use of induced mutagenesis to generate novel genetic variation is a prerequisite for crops with limited genetic variability, and hence methods are in place utilizing conventional and in vitro culture methods. In the horticulture sector, mutants for better plant architecture, novel flower, stress tolerance and improved yield have been developed. Large number of mutants in horticultural plants, especially ornamentals, has greatly contributed to the economy of several countries. In the present and future, there is an increasing demand for novel and improved horticultural plants (fruits, vegetables, and other horticultural commodities) to cater the needs of growing population. Horticultural plants need to be developed with better plasticity, stress tolerance, yield and other quality attributes under the climate change. There is a slow progress in genetic improvement of vegetable and fruit crops and hence greater efforts are required to induce mutations. Mutagenesis is also a feasible approach as it is devoid of the regulatory restrictions normally imposed on genetically modified plants. Transgenic technology has shown a considerable success for the improvement of horticultural plants for yield, disease resistance, flower color, flower longevity, and fragrances. Current transgenic research in horticultural crops is more focused on the development of cultivars to withstand biotic and abiotic stress besides nutritional quality and other value-added traits. Despite the success, commercialization of transgenic fruits, vegetables, nuts, and ornamentals is yet to come up in line to that of agronomic crops. In the coming years, induced mutations and transgenic methods will foster development of novel horticultural plants and commodities to meet the challenges posed by ever-growing human population and climate change.

Literature cited

- Ahloowalia, B.S., and Maluszynski, M. (2001). Induced mutations – a new paradigm in plant breeding. *Euphytica* 118 (2), 167–173 <https://doi.org/10.1023/A:1004162323428>.
- Ahloowalia, B.S., Maluszynski, M., and Nichterlein, K. (2004). Global impact of mutation-derived varieties. *Euphytica* 135 (2), 187–204 <https://doi.org/10.1023/B:EUPH.0000014914.85465.4f>.
- Ahmad, Z., Abu Hassan, A., Salleh, S., Ariffin, S., Shamsudin, S., and Basiran, M.N. (2012). Improvement of Malaysian ornamental plants through induced mutation. *Pertanika, J. Trop. Agric. Sci.* 35, 631–636.
- Ahuja, M.R., and Jain, S.M. (eds.). (2015). *Genetic Diversity and Erosion in Plants-Indicators and Prevention*. Volume 1 (Berlin, Heidelberg: Springer).
- Ahuja, M.R., and Jain, S.M. (eds.). (2016). *Genetic Diversity and Erosion in Plants-Case Histories*. Volume 2 (Berlin, Heidelberg: Springer).
- Ahuja, I., de Vos, R.C., Bones, A.M., and Hall, R.D. (2010). Plant molecular stress responses face climate change. *Trends Plant Sci.* 15 (12), 664–674 <https://doi.org/10.1016/j.tplants.2010.08.002>. PubMed

- Andrieu, A., Breitler, J.C., Siré, C., Meynard, D., Gantet, P., and Guiderdoni, E. (2012). An in planta, *Agrobacterium*-mediated transient gene expression method for inducing gene silencing in rice (*Oryza sativa* L.) leaves. *Rice* (N Y) 5 (1), 23 <https://doi.org/10.1186/1939-8433-5-23>. PubMed
- Ayub, R., Guis, M., Ben Amor, M., Gillot, L., Roustan, J.P., Latché, A., Bouzayen, M., and Pech, J.C. (1996). Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. *Nat. Biotechnol.* 14 (7), 862–866 <https://doi.org/10.1038/nbt0796-862>. PubMed
- Beddington, J.R., Asaduzzaman, M., Clark, M.E., Fernández Bremauntz, A., Guillou, M.D., Jahn, M.M., Lin, E., Mamo, T., Negra, C., Nobre, C.A., et al. (2012). The role for scientists in tackling food insecurity and climate change. *Agric. Food Secur.* 1 (1), 10 <https://doi.org/10.1186/2048-7010-1-10>.
- Bent, A.F. (2000). *Arabidopsis* in planta transformation. Uses, mechanisms, and prospects for transformation of other species. *Plant Physiol.* 124 (4), 1540–1547 <https://doi.org/10.1104/pp.124.4.1540>. PubMed
- Butelli, E., Titta, L., Giorgio, M., Mock, H.P., Matros, A., Peterek, S., Schijlen, E.G., Hall, R.D., Bovy, A.G., Luo, J., and Martin, C. (2008). Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nat. Biotechnol.* 26 (11), 1301–1308 <https://doi.org/10.1038/nbt.1506>. PubMed
- Chakrabarti, A., Ganapathi, T.R., Mukherjee, P.K., and Bapat, V.A. (2003). MSI-99, a magainin analogue, imparts enhanced disease resistance in transgenic tobacco and banana. *Planta* 216 (4), 587–596.
- Chandler, S. (2013). Genetically Engineered Ornamental Plants:Regulatory Hurdles to Commercialization (ISB News report), p.1–3.
- Chandler, S.F., and Sanchez, C. (2012). Genetic modification; the development of transgenic ornamental plant varieties. *Plant Biotechnol. J.* 10 (8), 891–903 <https://doi.org/10.1111/j.1467-7652.2012.00693.x>. PubMed
- Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* 16 (6), 735–743 <https://doi.org/10.1046/j.1365-313x.1998.00343.x>. PubMed
- Courtney-Gutterson, N., Napoli, C., Lemieux, C., Morgan, A., Firoozabady, E., and Robinson, K.E. (1994). Modification of flower color in florist's chrysanthemum: production of a white-flowering variety through molecular genetics. *Biotechnology (N.Y.)* 12 (3), 268–271 <https://doi.org/10.1038/nbt0394-268>. PubMed
- Datta, S.K. (2009). A Report on 36 Years of Practical Work on Crop Improvement Through Induced Mutagenesis. In *Induced Plant Mutations in the Genomics Era*, Q.Y. Shu, ed. (Rome: Food and Agriculture Organization of the United Nations), p.253–256.
- Datta, S.K. (2014). Induced mutagenesis: basic knowledge for technological success. In *Mutagenesis: Exploring Genetic Diversity of Crops*, N.B. Tomlekova, M.I. Kazgar, M.R. Wani, eds. (The Netherlands: Wageningen Academic Publishers), p.97–139.
- Debener, T., and Winkelmann, T. (2010). Ornamentals. In: *Genetic Modification of Plants* (Berlin, Heidelberg: Springer), p.369–391.
- Diretto, G., Al-Babili, S., Tavazza, R., Papacchioli, V., Beyer, P., and Giuliano, G. (2007). Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini-pathway. *PLoS ONE* 2 (4), e350 <https://doi.org/10.1371/journal.pone.0000350>. PubMed
- Dobres, M.S. (2011). Prospects for commercialisation of transgenic ornamentals. In *Transgenic Horticultural Crops; Challenges and Opportunities*, B. Mou, and R. Scorza, eds. (Boca Raton, FL: CRC Press), p.305–316.
- Elitzur, T., Yakir, E., Quansah, L., Zhangjun, F., Vrebalov, J., Khayat, E., Giovannoni, J.J., and Friedman, H. (2016). Banana *MaMADS* transcription factors are necessary for fruit ripening and molecular tools to promote shelf-life and food security. *Plant Physiol.* 171 (1), 380–391 <https://doi.org/10.1104/pp.15.01866>. PubMed
- FAO/IAEA MVDB. (2016). <http://www-mvd.iaea.org>.
- Fatima, T., Sobolev, A.P., Teasdale, J.R., Kramer, M., Bunce, J., Handa, A.K., and Mattoo, A.K. (2016). Fruit metabolite networks in engineered and non-engineered tomato genotypes reveal fluidity in a hormone and agroecosystem specific manner. *Metabolomics* 12, 103.
- Fuentes, A., Carlos, N., Ruíz, Y., Callard, D., Sánchez, Y., Ochagavía, M.E., Seguin, J., Malpica-López, N., Hohn, T., Lecca, M.R., et al. (2016). Field trial and molecular characterization of RNAi-transgenic tomato plants that exhibit resistance to tomato yellow leaf curl geminivirus. *Mol. Plant Microbe Interact.* 29 (3), 197–209 <https://doi.org/10.1094/MPMI-08-15-0181-R>. PubMed
- Fukui, Y., Tanaka, Y., Kusumi, T., Iwashita, T., and Nomoto, K. (2003). A rationale for the shift in colour towards blue in transgenic carnation flowers expressing the flavonoid 3',5'-hydroxylase gene. *Phytochemistry* 63 (1), 15–23 [https://doi.org/10.1016/S0031-9422\(02\)00684-2](https://doi.org/10.1016/S0031-9422(02)00684-2). PubMed
- García-Sogo, B., Pineda, B., Roque, E., Antón, T., Atarés, A., Borja, M., Beltrán, J.P., Moreno, V., and Cañas, L.A. (2012). Production of engineered long-life and male sterile Pelargonium plants. *BMC Plant Biol.* 12 (1), 156

<https://doi.org/10.1186/1471-2229-12-156>. PubMed

Ghag, S.B., Shekhawat, U.K.S., and Ganapathi, T.R. (2012). Petunia floral defensins with unique prodomains as novel candidates for development of fusarium wilt resistance in transgenic banana plants. *PLoS ONE* 7 (6), e39557 <https://doi.org/10.1371/journal.pone.0039557>. PubMed

Ghag, S.B., Shekhawat, U.K., and Ganapathi, T.R. (2014). Host-induced post-transcriptional hairpin RNA-mediated gene silencing of vital fungal genes confers efficient resistance against Fusarium wilt in banana. *Plant Biotechnol. J.* 12 (5), 541–553 <https://doi.org/10.1111/pbi.12158>. PubMed

Ghag, S.B., Shekhawat, U.K., and Ganapathi, T.R. (2015). Fusarium wilt of banana: biology, epidemiology and management. *Int. J. Pest Manage.* 61 (3), 250–263 <https://doi.org/10.1080/09670874.2015.1043972>.

Gonsalves, D. (2004). Sidebar: virus-resistant transgenic papaya helps save Hawaiian industry. *Calif. Agric.* 58, 92–93.

Gupta, A., Pal, R.K., and Rajam, M.V. (2013). Delayed ripening and improved fruit processing quality in tomato by RNAi-mediated silencing of three homologs of 1-aminopropane-1-carboxylate synthase gene. *J. Plant Physiol.* 170 (11), 987–995 <https://doi.org/10.1016/j.jplph.2013.02.003>. PubMed

Hallerman, E., and Grabau, E. (2016). Crop biotechnology: a pivotal moment for global acceptance. *Food Energy Secur.* 5 (1), 3–17 <https://doi.org/10.1002/fes3.76>.

Halterman, D.A., Kramer, L.C., Wielgus, S., and Jiang, J. (2008). Performance of transgenic potato containing the late blight resistance gene RB. *Plant Dis.* 92 (3), 339–343 <https://doi.org/10.1094/PDIS-92-3-0339>.

Hefferon, K.L. (2015). Nutritionally enhanced food crops; progress and perspectives. *Int J Mol Sci* 16 (2), 3895–3914 <https://doi.org/10.3390/ijms16023895>. PubMed

Jain, S.M. (2002). A review of induction of mutations in fruits of tropical and subtropical regions. *Acta Hort.* 575, 295–302 <https://doi.org/10.17660/ActaHortic.2002.575.33>.

Jain, S.M. (2005). Major mutation-assisted plant breeding programs supported by FAO/IAEA. *Plant Cell Tiss. Org. Cult.* 82 (1), 113–123 <https://doi.org/10.1007/s11240-004-7095-6>.

Jain, S.M. (2006). Mutation-assisted breeding for improving ornamental plants. *Acta Hort.* 714, 85–98 <https://doi.org/10.17660/ActaHortic.2006.714.10>.

Jain, S.M. (2010). In vitro mutagenesis in Banana (*Musa* spp.) improvement. *Acta Hort.* 879, 605–614 <https://doi.org/10.17660/ActaHortic.2010.879.67>.

Jain, S.M. (2012). In vitro mutagenesis for improving date palm (*Phoenix dactylifera* L.). *Emir. J. Food Agric.* 24, 400–407.

Jain, S.M., and Suprasanna, P. (2011). Induced mutations for enhancing nutrition for food production. *Gene Conserve* 40, 201–215.

Jain, S.M., Ochatt, S.J., Kulkarni, V.K., and Predieri, S. (2010). In vitro culture for mutant development. *Acta Hort.* 865, 59–68 <https://doi.org/10.17660/ActaHortic.2010.865.6>.

Jain, S.M., Till, B., Suprasanna, P., and Roux, N. (2011). Mutations and cultivar development of banana. In *Banana breeding-progress and challenges*, M. Pillay, and A. Tenkouano eds. (USA: CRC Press, Taylor and Francis), p.203–217

Jin, W., Dong, J., Hu, Y., Lin, Z., Xu, X., and Han, Z. (2009). Improved cold-resistant performance in transgenic grape (*Vitis vinifera* L.) overexpressing cold-inducible transcription factors *AtDREB1b*. *HortScience* 44, 35–39.

Jo, K.R., Kim, C.J., Kim, S.J., Kim, T.Y., Bergervoet, M., Jongsma, M.A., Visser, R.G., Jacobsen, E., and Vossen, J.H. (2014). Development of late blight resistant potatoes by cisgene stacking. *BMC Biotechnol.* 14 (1), 50 <https://doi.org/10.1186/1472-6750-14-50>. PubMed

Jones, J.D., Witek, K., Verweij, W., Jupe, F., Cooke, D., Dorling, S., Tomlinson, L., Smoker, M., Perkins, S., and Foster, S. (2014). Elevating crop disease resistance with cloned genes. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 369 (1639), 20130087 <https://doi.org/10.1098/rstb.2013.0087>. PubMed

Katavic, V., Haughn, G.W., Reed, D., Martin, M., and Kunst, L. (1994). In planta transformation of *Arabidopsis thaliana*. *Mol. Gen. Genet.* 245 (3), 363–370 <https://doi.org/10.1007/BF00290117>. PubMed

Kharkwal, M.C., and Shu, Q.Y. 2009. The role of induced mutations in world food security. In *Induced Plant Mutations in the Genomics Era*, Q.Y. Shu, ed. (Rome, Italy: Food and Agriculture Organization of the United Nations), p.33–38.

Koul, B., Srivastava, S., Sanyal, I., Tripathi, B., Sharma, V., and Amla, D.V. (2014). Transgenic tomato line expressing modified *Bacillus thuringiensis cry1Ab* gene showing complete resistance to two lepidopteran pests. *Springerplus* 3 (1), 84–96 <https://doi.org/10.1186/2193-1801-3-84>. PubMed



- Kovács, G., Sági, L., Jacon, G., Arinaitwe, G., Busogoro, J.P., Thiry, E., Strosse, H., Swennen, R., and Remy, S. (2013). Expression of a rice chitinase gene in transgenic banana ('Gros Michel', AAA genome group) confers resistance to black leaf streak disease. *Transgenic Res.* 22 (1), 117–130 <https://doi.org/10.1007/s11248-012-9631-1>. PubMed
- Krishna, V.V., and Qaim, M. (2008). Potential impacts of Bt eggplant on economic surplus and farmers' health in India. *Agric. Econ.* 38 (2), 167–180 <https://doi.org/10.1111/j.1574-0862.2008.00290.x>.
- Kume, T., Amano, E., Nakanishi, T.M., and Chino, M. (2002). Economic scale of utilization of radiation (II): agriculture comparison between Japan and the USA. *J. Nucl. Sci. Technol.* 39 (10), 1106–1113 <https://doi.org/10.1080/18811248.2002.9715300>.
- Lal, R. (2004a). Carbon emission from farm operations. *Environ Int* 30 (7), 981–990 <https://doi.org/10.1016/j.envint.2004.03.005>. PubMed
- Liu, L., Guo, H., Zhao, L., and Zhao, S. 2007a. Advances in induced mutations for crop improvement in China. Paper presented at: China-Korea Joint Symposium on Nuclear Technique Application in Agriculture and Life Science (Hangzhou, China).
- Liu, L., Guo, H., Zhao, L., Gu, J., and Zhao, S. (2007b). Achievements in the past twenty years and perspective outlook of crop space breeding in China. *He-Nong Xuebao* 21 (6), 589–592.
- Lu, C.Y., Chandler, S.F., Mason, J.G., and Brugliera, F. (2003). Florigene flowers: From laboratory to market. In *Plant Biotechnology 2002 and Beyond*, I.K. Vasil, ed. (Dordrecht, The Netherlands: Kluwer Academic), p.333–336.
- Maluszynski, M., Szarejko, I., and Maluszynska, J. (2004). Mutation techniques. *Encycl. Appl. Plant Sci.* 1–3, 186–201.
- Mba, C., Afza, R., Jankowicz-Cieslak, J., Bado, S., Matijevic, M., Huynh, O., and Till, B.J. (2009). Enhancing genetic diversity through induced mutagenesis in vegetatively propagated plants. In *Induced Plant Mutations in the Genomics Era*, Q.Y. Shu ed. (Rome: Food and Agriculture Organization of the United Nations), p.262–265
- Meli, V.S., Ghosh, S., Prabha, T.N., Chakraborty, N., Chakraborty, S., and Datta, A. (2010). Enhancement of fruit shelf life by suppressing N-glycan processing enzymes. *Proc. Natl. Acad. Sci. U.S.A.* 107 (6), 2413–2418 <https://doi.org/10.1073/pnas.0909329107>. PubMed
- Meyer, P., Heidmann, I., Forkmann, G., and Saedler, H. (1987). A new petunia flower colour generated by transformation of a mutant with a maize gene. *Nature* 330 (6149), 677–678 <https://doi.org/10.1038/330677a0>. PubMed
- Mou, B., and Scorza, R. (2011) *Transgenic Horticultural Crops: Challenges and Opportunities* (Boca Raton, USA: CRC).
- Murata, Y., Itoh, Y., Iwashita, T., and Namba, K. (2015). Transgenic petunia with the iron(III)-phytosiderophore transporter gene acquires tolerance to iron deficiency in alkaline environments. *PLoS ONE* 10 (3), e0120227 <https://doi.org/10.1371/journal.pone.0120227>. PubMed
- Nichol, N.L., Zarka, K.A., Coombs, J.J., and Douches, D.S. (2015). Field assessment of AtCBF1 transgenic potato lines (*Solanum tuberosum*) for drought tolerance. *Am. J. Potato Res.* 92 (2), 243–249 <https://doi.org/10.1007/s12230-014-9424-6>.
- Nishihara, M., and Nakatsuka, T. (2010). Genetic engineering of novel flower colors in floricultural plants: recent advances via transgenic approaches. *Methods Mol. Biol.* 589, 325–347 https://doi.org/10.1007/978-1-60327-114-1_29. PubMed
- Olsen, A., Lütken, H., Hegelund, J.N., and Müller, R. (2015). Ethylene resistance in flowering ornamental plants - improvements and future perspectives. *Hortic Res* 2, 15038 <https://doi.org/10.1038/hortres.2015.38>. PubMed
- Parry, M.A., Madgwick, P.J., Bayon, C., Tearall, K., Hernandez-Lopez, A., Baudo, M., Rakszegi, M., Hamada, W., Al-Yassin, A., Ouabbou, H., et al. (2009). Mutation discovery for crop improvement. *J. Exp. Bot.* 60 (10), 2817–2825 <https://doi.org/10.1093/jxb/erp189>. PubMed
- Pérez-Massot, E., Banakar, R., Gómez-Galera, S., Zorrilla-López, U., Sanahuja, G., Arjó, G., Miralpeix, B., Vamvaka, E., Farré, G., Rivera, S.M., et al. (2013). The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. *Genes Nutr* 8 (1), 29–41 <https://doi.org/10.1007/s12263-012-0315-5>. PubMed
- Randhawa, G.J., Sharma, R., and Singh, M. (2012). Qualitative and event-specific real-time PCR detection methods for Bt brinjal event EE-1. *J AOAC Int* 95 (6), 1733–1739 <https://doi.org/10.5740/jaoacint.11-478>. PubMed
- Ryuto, H., Fukunishi, N., Hayashi, Y., Ichida, H., Abe, T., Kase, M., and Yano, Y. (2008). Heavy-ion beam irradiation facility for biological samples in RIKEN. *Plant Biotechnol.* 25 (1), 119–122 <https://doi.org/10.5511/plantbiotechnology.25.119>.
- Schaffer, R.J., Ireland, H.S., Ross, J.J., Ling, T.J., and David, K.M. (2013). SEPALLATA1/2-suppressed mature apples

have low ethylene, high auxin and reduced transcription of ripening-related genes. *AoB Plants* 5 (0), pls047 <https://doi.org/10.1093/aobpla/pls047>. PubMed

Scorza, R., Callahan, A., Dardick, C., Ravelonandro, M., Polak, J., Malinowski, T., Zagrai, I., Cambra, M., and Kamenova, I. (2013). Genetic engineering of Plum pox virus resistance: 'HoneySweet' plum—from concept to product. *Plant Cell Tiss. Org. Cult.* 115 (1), 1–12 <https://doi.org/10.1007/s11240-013-0339-6>.

Shekhawat, U.K.S., Ganapathi, T.R., and Srinivas, L. (2011). Cloning and characterization of a novel stress-responsive WRKY transcription factor gene (*MusaWRKY71*) from *Musa* spp. cv. Karibale Monthan (ABB group) using transformed banana cells. *Mol. Biol. Rep.* 38 (6), 4023–4035 <https://doi.org/10.1007/s11033-010-0521-4>. PubMed

Shekhawat, U.K., Ganapathi, T.R., and Hadapad, A.B. (2012). Transgenic banana plants expressing small interfering RNAs targeted against viral replication initiation gene display high-level resistance to banana bunchy top virus infection. *J. Gen. Virol.* 93 (Pt 8), 1804–1813 <https://doi.org/10.1099/vir.0.041871-0>. PubMed

Shu, Q.Y. (2009) Turning plant mutation breeding into a new era: Molecular mutation breeding. In *Induced Plant Mutations in the Genomics Era*, Q.Y. Shu, ed. (Rome: Food and Agriculture Organization of the United Nations), p.425–427

Suprasanna, P., and Nakagawa, H. (2012). Mutation breeding of vegetatively propagated crops. In *Plant Mutation Breeding and Biotechnology* (Food and Agriculture Organization of the United Nations), p.347–358.

Suprasanna, P., Mirajkar, S.J., and Bhagwat, S.G. (2015). Induced mutations and crop improvement. In *Plant Biology and Biotechnology*, B. Bahadur, M.V. Rajam, L. Sahijram, and K.V. Krishnamurthy, eds. (India: Springer), p.593–617

Tecson Mendoza, E.M., C Laurena, A., and Botella, J.R. (2008). Recent advances in the development of transgenic papaya technology. *Biotechnol Annu Rev* 14, 423–462 [https://doi.org/10.1016/S1387-2656\(08\)00019-7](https://doi.org/10.1016/S1387-2656(08)00019-7). PubMed

Trieu, A.T., Burleigh, S.H., Kardailsky, I.V., Maldonado-Mendoza, I.E., Versaw, W.K., Blaylock, L.A., Shin, H., Chiou, T.J., Katagi, H., Dewbre, G.R., et al. (2000). Transformation of *Medicago truncatula* via infiltration of seedlings or flowering plants with *Agrobacterium*. *Plant J.* 22 (6), 531–541 <https://doi.org/10.1046/j.1365-313x.2000.00757.x>. PubMed

Tripathi, S., Suzuki, J., and Gonsalves, D. (2007). Development of genetically engineered resistant papaya for papaya ringspot virus in a timely manner: a comprehensive and successful approach. *Methods Mol. Biol.* 354, 197–240. PubMed

Waltz, E. (2014). Vitamin A super banana in human trials. *Nat. Biotechnol.* 32 (9), 857–857 <https://doi.org/10.1038/nbt0914-857>. PubMed

Wilson, A.K., Latham, J.R., and Steinbrecher, R.A. (2006). Transformation-induced mutations in transgenic plants: analysis and biosafety implications. *Biotechnol. Genet. Eng. Rev.* 23 (1), 209–237 <https://doi.org/10.1080/02648725.2006.10648085>. PubMed

Xiong, J.-S., Ding, J., and Li, Y. (2015). Genome-editing technologies and their potential application in horticultural crop breeding. *Hortic Res* 2, 15019 <https://doi.org/10.1038/hortres.2015.19>. PubMed

